ImmunoTools *special* Award 2015

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Investigating the roles of macrophages and dendritic cells in liver fluke infection

Liver fluke infection caused by *Opisthorchis viverrini*, *O. felineus*, and *Clonorchis sinensis* is a major public health problem in East Asia and Eastern Europe. Currently, more than 600 million people are at risk of infection with these trematodes. *O. viverrini* is endemic in Southeast Asian countries, including Thailand, Lao People's Democratic Republic, Vietnam, and Cambodia, and *C. sinensis* infection is common in rural areas of Korea and China. Opisthorchiasis has been extensively studied in Thailand, where an estimated 6 million people are infected with the liver fluke (calculated from overall 9.4% prevalence within the population in 2001)

Most people with opisthorchiasis have no symptoms. Only 5%–10% of infected people, in general those with heavy fluke infections, have non-specific symptoms such as right upper quadrant abdominal pain, flatulence, and fatigue. Enlargement of the gall bladder can be detected by ultrasonography, and is reversed after elimination of flukes by praziquantel. Nonetheless, heavy, long-standing infection is associated with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, fibrosis of the periportal system, cholecystitis, and cholelithiasis. Moreover, both experimental and epidemiologic evidence strongly implicates liver fluke infection in the aetiology of one of the liver cancer subtypes—cholangiocarcinoma (CCA), or cancer of the bile ducts

Macrophages are found in close proximity with collagen-producing myofibroblasts and indisputably play a key role in fibrosis. Fibrosis results when normal wound-healing responses persist or are not regulated properly, usually in response to some type of repeated injury. For example, the primary causes of liver fibrosis include persistent hepatitis C virus infection and chronic infections with the helminth parasites. Recent studies have also identified macrophages as critical regulators of fibrosis.

Hence, our project we hypothesized that macrophages from individuals may have different capacity to either promote or persist fibrosis. Since macrophage is the key player in early formation of fribosis, we will use antibodies markers such as CD14, CD45, CD80 and CD163 to quantitate absolute macrophages numbers from patients blood sample. Because of low number of macrophages found in peripheral blood, we will use cytokines such as GM-CSF, IL-4, M-CSF and Flt-3L to differentiate macrophages from peripheral blood monocytes.

Furthermore, macrophages are considered as professional antigen presenting cells also play a key role in presenting antigen to T lymphocytes. We will also investigate the macrophages from individual patients on their capacity to present *Opisthorchis viverrini* antigen to both $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes. Here, we will employ flow cytometry to look for T cell activation markers such as CD25, CD44, CD69 and also intracellular IFNγ to quantitate numbers of activated T cells.

Apart from macrophages, we also have strong interests in another innate immune cells such as dendritic cells. Similar to macrophages, we will characterise dendritic cells costimulatory molecules such as CD40, CD80, CD86, MHCII, MHCI after cell maturation upon *Opisthorchis viverrini* antigen encounter.